

Listing of Claims

1. (Previously Presented) A method, comprising:
synthesizing one or more nucleic acid sequences, each relevant for use as a clinical reference;
tagging at least one end of each sequence for amplification by a primer; and
amplifying the one or more nucleic acid sequences using the primer.
2. (Previously Presented) The method as recited in claim 1, wherein the tagging includes attaching an additional sequence of nucleotides, wherein the additional sequence is complementary or identical to a nucleotide sequence of the primer.
3. (Canceled)
4. (Original) The method as recited in claim 1, wherein:
the tagging includes attaching a first sequence of nucleotides to a first end of each of the one or more synthesized nucleic acid sequences, wherein the first sequence is complementary to a nucleotide sequence of a first primer of a primer set, and
the tagging includes attaching a second sequence of nucleotides to a second end of each of the one or more synthesized nucleic acid sequences,

wherein the second sequence is identical to a nucleotide sequence of a second primer of a primer set.

5. (Original) The method as recited in claim 1, wherein the synthesizing comprises synthesizing two complementary nucleic acid strands, wherein:

a first strand includes a first nucleic acid sequence relevant for clinical reference and a nucleic acid tag complementary to a first primer of a primer set and

a second strand includes a nucleic acid sequence complementary to the first strand and a nucleic acid tag complementary to a second primer of a primer set.

6. (Canceled)

7. (Original) The method as recited in claim 1, wherein at least one of the one or more synthesized nucleic acid sequences includes at least one mutation of a nucleotide in a normal human nucleic acid.

8. (Original) The method as recited in claim 1, further comprising synthesizing multiple mixtures of at least one reference nucleic acid apiece, wherein:

each of the multiple mixtures has an associated primer set, and wherein:

each member of one of the multiple mixtures includes a first tag attached to a first end of the member, wherein:

the first tag comprises a sequence of nucleotides complementary to a nucleotide sequence of a first primer of the associated primer set, and

each member includes a second tag attached to a second end of the member, wherein:

the second tag comprises a sequence of nucleotides identical to a nucleotide sequence of a second primer of the associated primer set.

9. (Previously Presented) The method as recited in claim 8, further comprising combining each of the multiple mixtures with each other and separately controlling each of the multiple mixtures to achieve separate amounts of amplification for each of the multiple mixtures components.

10. (Canceled)

11. (Previously Presented) The method as recited in claim 9, wherein separately controlling each of the multiple mixtures includes controlling a physical characteristic of a combined mixture of the multiple mixtures to favor an amplification capability of one primer set over an amplification capability another primer set.

12-15 (Canceled)

16. (Original) The method as recited in claim 1, further comprising adding normal human nucleic acid to the one or more synthesized nucleic acid sequences relevant for clinical reference in order to achieve a mixture of the nucleic acids representing at least a segment of homologous heterozygous alleles.

17-20 (Canceled)

21. (Previously Presented) The method as recited in claim 1, further comprising joining multiple nucleic acid segments using a ligation extension to perform the synthesizing one or more reference nucleic acid sequences.

22. (Original) The method as recited in claim 21, wherein for at least one of the reference nucleic acids, the synthesizing includes:

synthesizing a first nucleic acid that includes a first end comprising a base sequence complementary to the base sequence of the first primer and a second end complementary to a base sequence on a first end of a bridge nucleic acid;

synthesizing a second nucleic acid that includes a first end comprising a base sequence that matches the base sequence of the second

primer and a second end complementary to a second end of the bridge nucleic acid; and

making the reference nucleic acid by joining multiple nucleic acid segments in the ligation extension, including joining the first nucleic acid on one end of the joined segments using the bridge nucleic acid and joining the second nucleic acid on the opposite end of the joined segments using the bridge nucleic acid.

23. (Previously Presented) The method as recited in claim 1[[7]], further comprising joining multiple nucleic acids using an overlap extension to perform the synthesizing one or more reference nucleic acid sequences.

24 -27 (Canceled)

28. (Previously Presented) The method as recited in claim 1, wherein the synthesizing and the tagging include a ligation extension of two or more nucleic acids.

29-50 (Canceled)

51. (Withdrawn) A tagged reference nucleic acid for a polymerase chain reaction amplification, comprising:

a synthesized reference nucleic acid having a base sequence capable of being used as a reference;

a first nucleic acid tag bound to a first end of the synthesized reference nucleic acid, wherein the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer set; and

a second nucleic acid tag bound to a second end of the synthesized reference nucleic acid, wherein the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

52. (Withdrawn) The tagged reference nucleic acid as recited in claim 51, wherein the synthesized reference nucleic acid includes a base sequence representing a mutation of a gene.

53. (Withdrawn) The tagged reference nucleic acid as recited in claim 52, wherein the gene comprises a cystic fibrosis transmembrane conductance regulator gene.

54-70 (Canceled)

71. (Original) A method, comprising:
synthesizing a first mixture of various reference nucleic acids, wherein each of the various reference nucleic acids in the first mixture

includes one or more tags allowing PCR amplification of the first mixture via a primer set specific to the tags of the first mixture; and

synthesizing a second mixture of various reference nucleic acids, wherein each of the various reference nucleic acids in the second mixture includes one or more tags allowing PCR amplification of the second mixture via a second primer set specific to the tags of the second mixture.

72. (Original) The method as recited in claim 71, further comprising combining the first and second mixtures to make a single mixture and differentially amplifying the first mixture and the second mixture in a PCR reaction by controlling amounts of the first primer set and the second primer set in the single mixture.

73. (Original) The method as recited in claim 72, wherein at least some of the reference nucleic acids include mutations of a normal human nucleic acid.

74. (Original) The method as recited in claim 73, further comprising adding normal human nucleic acid to the single mixture to obtain heterozygous pairs, wherein each heterozygous pair includes a normal segment of human nucleic acid and a mutated copy of the normal segment of human nucleic acid.